Environmental Estrogens Alter Early Development in *Xenopus laevis*

Cassandra L. Bevan,[1] Donna M. Porter,[1] Anita Prasad,[1] Marthe J. Howard,[2] and Leslie P. Henderson[1]

[1]Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire, USA; [2]Department of Anatomy and Neurobiology, Medical College of Ohio, Toledo, Ohio, USA

A growing number of environmental toxicants found in pesticides, herbicides, and industrial solvents are believed to have deleterious effects on development by disrupting hormone-sensitive processes. We exposed *Xenopus laevis* embryos at early gastrula to the commonly encountered environmental estrogens nonylphenol, octylphenol, and methoxychlor, the antiandrogen, *p,p′*-DDE, or the synthetic androgen, 17α-methyltestosterone at concentrations ranging from 10 nM to 10 μM and examined them at tailbud stages (~48 hr of treatment). Exposure to the three environmental estrogens, as well as to the natural estrogen 17β-estradiol, increased mortality, induced morphologic deformations, increased apoptosis, and altered the deposition and differentiation of neural crest–derived melanocytes in tailbud stage embryos. Although neural crest–derived melanocytes were markedly altered in embryos treated with estrogenic toxicants, expression of the early neural crest maker *Xslug*, a factor that regulates both the induction and subsequent migration of neural crest cells, was not affected, suggesting that the disruption induced by these compounds with respect to melanocyte development may occur at later stages of their differentiation. Co-incubation of embryos with the pure antiestrogen ICI 182,780 blocked the ability of nonylphenol to induce abnormalities in body shape and in melanocyte differentiation but did not block the effects of methoxychlor. Our data indicate not only that acute exposure to these environmental estrogens induces deleterious effects on early vertebrate development but also that different environmental estrogens may alter the fate of a specific cell type via different mechanisms. Finally, our data suggest that the differentiation of neural crest–derived melanocytes may be particularly sensitive to the disruptive actions of these ubiquitous chemical contaminants. *Key words:* antiandrogens, apoptosis, embryogenesis, environmental toxicants, estrogens, melanocytes, neural crest, *Xenopus laevis*, *Xslug*. *Environ Health Perspect* 111:488–496 (2003). doi:10.1289/ehp.5500 Available via *http://dx.doi.org/* [Online 28 October 2002]

For nearly half a century, studies of both natural and laboratory vertebrate populations have suggested that exposure to a variety of environmental chemicals, including pesticides, herbicides, and industrial solvents, elicits deleterious effects during development by interfering with hormone-sensitive processes (Colborn et al. 1993). However, concern over the potentially harmful effects of these compounds has escalated within the past decade, spurred on by data demonstrating that more than 100,000 chemicals are now being produced on an industrial scale and several thousand new chemicals are introduced each year (Younes 1999). Some of the most prevalent and persistent of these compounds include the pesticide 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane (DDT) and its major metabolite, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl) ethylene (*p,p′*-DDE; Kelce et al. 1998; Sohoni and Sumpter 1998); methoxychlor, an analog of DDT (Palanza et al. 1999); and nonylphenol and octylphenol, degradation products of the alkylphenol polyethoxylates (APEOs). These APEOs are widely used as nonionic surfactants in commercial production as well as in herbicides, pesticides, polystyrene plastics, and paints (Cooper and Kavlock 1997; Sonnenschein and Soto 1998; White et al. 1994). Although contamination by these environmental toxicants is often first evident in the water supply, most of these compounds are highly lipophilic and bioaccumulate in fatty tissue, thereby presenting potential developmental hazards for both aquatic and terrestrial species (Crews et al. 2000; Menditto and Turrio-Baldassarri 1999; Sonnenschein and Soto 1998).

The majority of environmental toxicants known to interfere with hormone signaling, including nonylphenol and octylphenol, are believed to exert their effects at nuclear estrogen receptors (ERα or ERβ; Mueller and Kim 1978; White et al. 1994). However, recently it has been shown that some compounds, including *p,p′*-DDE, are devoid of action at the ER, but block signaling mediated by the androgen receptor (Kelce et al. 1998; Kelce and Gray 1999; Sohoni and Sumpter 1998). Finally, some environmental estrogens, including the major metabolite of methoxychlor, 2,2-*bis*(*p*-hydroxyphenyl)-1,1,1-trichloroethane, have been shown not only to be active at the ER [for review, see Cummings (1997)] but also to elicit biologic actions as an antiandrogen via nuclear hormone-independent mechanisms (Ghosh et al. 1999; Ren et al. 1997; Waters et al. 2001).

There is a wealth of data on the effects of exposure of environmental toxicants believed to interfere with hormone-sensitive processes related to reproductive development and sexual differentiation (Crews et al. 2000; Gray 1992; Kelce and Gray 1999; Sharara et al. 1998), but few studies have examined the effects of early exposure to these environmental toxicants on other aspects of vertebrate development. Because of their rapid development and their aquatic nature, amphibians may be particularly sensitive and useful sentinels for studying the effects of environmental toxicants on early development (Blaustein et al. 1994; Kloas et al. 1999). In particular, the laboratory frog *Xenopus laevis* provides an excellent model system to assess the effects of early exposure to environmental toxicants because of its ability to generate embryos on a daily basis and because the molecular and organismal development of this vertebrate has been described extensively (for review, see Mayor et al. 1999; Spitzer and Ribera 1998; Weinstein and Hemmati-Brivanlou 1999). A recent study (Mann and Bidwell 2000) published during the course of the present experiments showed that chronic exposure (~96 hr) of *Xenopus* embryos to the nonylphenol polyethoxylate (NPEO), Teric GN8, at moderate concentrations (EC_{50} = 2.8–4.6 mg/L; ~5–8 μM assuming a standard oligomer length of 8) induced malformations in *Xenopus* tadpoles including cardiac edema, microphthalmia, and improper gut coiling in embryos examined at stage 46 (Nieuwkoop and Faber 1967) and increased mortality by stages 39–40 in embryos exposed to higher concentrations (6.0–10 mg/L; 10–17 μM).

The effects reported for exposure to this synthetic compound mirror those produced by the naturally occurring estrogen 17β estradiol) (E_2) (Nishimura et al. 1997), which is consistent with a common mechanism of action for NPEO and E_2. Specifically, Nishimura et al. (1997) demonstrated that exposure (beginning at stage 3) to 10 μM E_2 caused increases in mortality and increased incidence of malformations, including crooked vertebrae, swollen stomachs, small eyes and heads, and suppressed organogenesis

Address correspondence to L.P. Henderson, Dept. of Physiology, Dartmouth Medical School, Hanover, NH 03755 USA. Telephone: (603) 650-1312. Fax: (603) 650-1128. E-mail:leslie.henderson@dartmouth.edu

We thank M. Bronner-Fraser for generously providing the *Xslug* plasmid, M. McPeek for access to and assistance with statistical programs, and A. Clark for critical review of the manuscript.

This research was supported by the National Institutes of Health (NIH) (ES10143 to L.P. Henderson and NS40644 to M.J. Howard). Support for C.L. Bevan was provided by T32DK07508 and ES10143 from NIH.

Received 25 January 2002; accepted 23 July 2002.